Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/18290
DOI URL: http://dx.doi.org/10.21474/IJAR01/18290

RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF ADENOVIRUS AND ROTAVIRUS ASSOCIATED DIARRHOEA AMONG UNDER-FIVES IN TWO TERTIARY HOSPITALS IN SOUTHERN NIGERIA

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Manuscript Info

Manuscript History
Received: 15 December 2023
Final Accepted: 18 January 2024
Published: February 2024

Key words:-
Adenovirus, Rotavirus, Diarrhoea, Accession Number

Abstract

Background: Viral diarrhoea continues to be a challenge among children under five years of age in Nigeria. While other non-viral agents of diarrhoea have been well documented, the agents of viral diarrhoea especially their genetic diversity have not been well studied in our locality, hence this study.

Method: This was a cross-sectional study carried out at the University of Benin Teaching Hospital (UBTH) and NnamdiAzikiwe University Teaching Hospital (NAUTH) in Benin City and Nnewi respectively. Three hundred and thirty participants were recruited for the study using stratified random sampling, aged between 1 and 5 years. All the diarrhoeic stools had their DNA/RNA extracted using the Quick-RNA Viral MiniPrep Kit and stored at -80°C for polymerase chain reaction. Sequences of the adenovirus were done using a Big Dye DNA sequencing kit. Bioinformatics was done using R packages in R studio v3 software. Data from the study were analyzed using chi-square and level of significance expressed using p-value ($p<0.05$).

Results: Adenoviral diarrhoea was higher in Benin City (18; 11.8%) than Nnewi (11; 6.1%). Females (55.2%) were more infected than the males (44.8%) ($p>0.05$). Among the population studied, there was no rotavirus genome detected. Ten (10) adenovirus sequences from serotype 40 were identified and deposited into the National Centre for Biotechnology Information (Genbank: http://www.ncbi.nlm.nih.gov/) under the accession number ON128719 - ON128728. The prevalence of adenovirus in the study locality is 8.7%. More females were infected than the males, although not statistically significant ($p>0.05$).

Conclusion: The study also concluded that sex had no relationship with the spread of adenovirus diarrhoea among under-fives ($p>0.05$).

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Introduction:-

Diarrhoeal disease is a leading cause of morbidity and mortality globally in children under five years of age, especially in developing countries like Nigeria (Mathew et al., 2023; Meyer et al., 2015). Enteric viruses are the major aetiologic agents of diarrhoea or acute gastroenteritis among infants and young children worldwide. The commonly implicated aetiologic agents are rotavirus, norovirus, adenovirus, and astrovirus (Rossouw et al., 2021). The World Health Organization (WHO) data showed that each child practically has viral diarrhoea irrespective of race and socioeconomic status within the first 5 years of life and this has great economic burden for public health services and the entire society (Imade and Eghafona, 2015; Ugboko et al., 2020).

Rotavirus infection is the leading cause of severe acute diarrhoea among young children worldwide (Crawford et al., 2017) and has been reported to be responsible for endemic viral diarrhoea in children in Nigeria (Mathew et al., 2023). Rotavirus and adenovirus have been identified as the leading viral agents responsible for diarrhoea in children who are under five years of age (Rossouw et al., 2021; Smith and Ajayi, 2017). Several factors such as poor hygiene and sanitation, feeding children with leftover foods and limited access to safe drinkingwater, have been implicated in the spread of viral diarrhoea. Others, such as inadequate education of health care providers and recipients have also been identified as possible factors causing the unabated spread of these viral agents to children less than 5 years of age (Birhan et al., 2018).

Despite reports from other African countries that there has been significant decline in the prevalence of rotavirus since the introduction of the Rotarix vaccine patented by Glaxosmithkline (Smith and Ajayi, 2017), there has been no such report from search of available literature in Nigeria. Also, while other non-viral agents of diarrhoea have been well documented, the agents of viral diarrhoea especially their genetic diversity have not been well studied in our locality.

Methods:-

Sample collection and processing

A total of three hundred and thirty samples (330) diarrhoeic stool samples were collected using sterile wide mouth container for this study. This comprised one hundred and seventy nine (179) from the NnamdiAzikiwe University Teaching Hospital (NAUTH) and one hundred and fifty three (153) from the University of Benin Teaching Hospital (UBTH). Prior to commencing this study, necessary ethical approvals were obtained from the study locations. The samples were then processed using the method described by (Jijoho et al., 2018), and the stool sample suspensions were stored briefly prior to extraction of genomic materials, using an RNA/DNA guard (Zymo Research) for rotavirus and adenovirus respectively to ensure the genetic materials were not lost during storage. All the diarrhoeic stools had their DNA/RNA extracted using the Quick-RNA Viral MiniPrep Kit (Zymo Research) according to the manufacturer's instructions and the extracted DNA/RNA were stored directly at -80°C until polymerase chain reaction using polymerase chain reaction (PCR).

Conversion of dsRNA to cDNA

The conversion of dsRNA to cDNA was done using the method of (Gouvea et al., 1990). Using this method, the dsRNA extracted from the stool samples were used as templates for reverse transcriptase to synthesize cDNAcopies

Table I:- Primers for adenovirus and rotavirus amplification.

Organism	Primer name/Sequence 5'-3'	Size of primer	Reference
Rotavirus	**VP7**: F con3 TGG CTT CGC TCA TTT ATA GAC A R con2 ATT TCG GAC CAT TTA TAA CC	1500bp	Magzoubet al., 2017
Adenovirus	**AdV F**: GCC ACG GTG GGG TTT CTA AAC TT **AdV R**: GCCCCAGTGGTCTTACATGCACATC	131bp	Meryemet al., 2017

from both viral strands that were then amplified by PCR. Oligonucleotide primers were derived from published nucleotide sequences as shown in Table 1. These primers were observed from previous studies to be corresponding to sequences on the template molecule, hence their choice of use in this study (Table i).

Polymerase chain reaction
All extracted cDNA and DNA were then subjected to polymerase chain reaction on the Applied Biosystems 2720 and GeneAmp PCR sytem 2400 in a 50µl reaction respectively typically following the denaturation (94^0C), annealing (52^0C) and elongation (72^0C) stages. For rotavirus, the process was optimized to increase the chances of getting many amplicons and elongation temperature was reduced to 68^0C.

Agarose gel preparation and electrophoresis
One gram (1g) of agarose powder (Ambion, USA) was weighed and dissolved by heating in 100 ml 1 x TBE buffer (AppliChem) for 3-5 minutes in a microwave. Then it was allowed to cool to 56^0C in a water bath. Thereafter 6µl of 100mg/ml ethidium bromide was added, mixed and poured on to the casting tray that had been trapped up appropriately and equipped with suitable comb to form well in the plate. The gel was allowed to set at room temperature. After 45 minutes of solidification, the comb was gently removed and the spacer from the open sides was removed as well. This made a 1% (w/v) agarose gel

A ten microlitre (10µl) volume of the PCR amplicons (products) was electrophoresed on a 1% agarose gel made in Tris acetated EDTA buffer (pH=8.0-8.5) using a nanoPAC 300 electrophoresis and visualized by UV illumination after ethidium bromide (10 g/ml) staining on peQLab 12200851 GeneCapture machine. PCR amplicons showing bands of 1500bp and 131bp thereafter were considered positive for rotavirus and adenovirus respectively.

Sequencing, phylogenetics and bioinformatics
Using the same primer sets used for PCR, the PCR products were sequenced using a Big Dye DNA sequencing kit in an automated DNA sequencer, an ABI Prism 3130 XL Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences obtained were compared with the National Centre for Biotechnology Information (NCBI) GenBank database to determine the adenovirus serotypes. Nucleotide sequence alignment was also performed using Clustal W multiple alignment. Phylogenetic analysis was constructed using the UgeneUnipro v44.The nucleotide sequences obtained in this study were deposited in GenBank under accession numbers. Data were captured using ABI 3730 software, and analyzed using R packages in Rstudio v3 software. Data obtained from this study were captured and validated using descriptive statistics such as mean, standard deviation, frequency; chi square and p-value were used to describe the socio-demographic characteristics of subjects and proportion of positive tests at 95% confidence interval.

Results:-

Twenty nine (29) of the 330 stool samples were positive for adenovirus diarrhoea constituting an overall prevalence of 8.7%. More females (16; 55.2%) were positive for adenovirus than their male counterparts (13; 44.8%) among the population studied. None of these samples was positive for rotavirus diarrhoea using the polymerase chain reaction (PCR) technique. In the study locations, sex had no statistically significant effect on the occurrence of adenovirus or rotavirus diarrhoea in the under-fives ($p > 0.05$; $\chi^2 = 5.02$) as shown in Table ii.

Table II:-Prevalence of Adenovirus and Rotavirus diarrhea among under-fives at NAUTH, Nnewi and UBTH, Benin City.

Study sites	No. of samples	No. positive		Sex				Total (%)
		Rota	Adeno	Male		Female		
NAUTH	179	0	11	0	2	0	9	11 (6.1)
UBTH	151	0	18	0	11	0	7	18 (11.8)
Total (%)		0	29 (8.7)	0	13 (44.8)	0	16 (55.2)	
$\chi^2 = 5.02$, $p > 0.05$								

PCR = polymerase chain reaction, Rota = rotavirus, Adeno = adenovirus, NAUTH = NnamdiAzikiwe University Teaching Hospital, UBTH = University of Benin Teaching Hospital

A total of ten (10) adenovirus sequences were obtained from this study and were deposited into the National Centre for Biotechnology Information (Genbank: http://www.ncbi.nlm.nih.gov/)under the accession number ON128719 -

ON128728. Ninety percent of the adenovirus sequences had close relationship with close relatives in the Genbank (Table iii).

Table III:-The human adenovirus identification, accession numbers and their close relatives in NCBI from under-fives in NAUTH, Nnewi and UBTH, Benin City.

No	Sample ID	Location	Isolate	Accession number	Similarity to close relatives in NCBI (%)	Accession no of close relatives in NCBI
1	1_Alfred-Adeno_B05_05	UBTH	Human Adenovirus sp.	ON128719	100	MK995001.1
2	2_Alfred-Adeno_C12_09	NAUTH	Human Adenovirus sp.	ON128720	98	MF962525.1
3	3_Alfred-Adeno_D05_11	NAUTH	Human Adenovirus sp.	ON128721	98	MF962521.1
4	4_Alfred-Adeno_E05_14	NAUTH	Human Adenovirus sp.	ON128722	98	MF962520.1
5	5_Alfred-Adeno_F05_17	NAUTH	Human Adenovirus sp.	ON128723	98	MF962511.1
6	6_Alfred-Adeno_D12_12	UBTH	Human Adenovirus sp.	ON128724	98	MF962509.1
7	7_Alfred-Adeno_H05_23	UBTH	Human Adenovirus sp.	ON128725	98	MF962499.1
8	8_Alfred-Adeno_A06_03	NAUTH	Human Adenovirus sp.	ON128726	98	MF962498.1
9	9_Alfred-Adeno_B06_06	NAUTH	Human Adenovirus sp.	ON128727	98	MF962494.1
10	10_Alfred-Adeno_C06_09	UBTH	Human Adenovirus sp.	ON128728	98	MF962492.1

NCBI = National Centre for Biotechnology Information, ON = Hexon protein, ON128719 (ON = Locus name, 128719 = accession number), MK995001.1 (MK = Locus name, 995001.1 = accession number and version)

The sequences had pairwise nucleotide similarity and the sequence lengths were all annotated as hexon protein genes (Table iv).

Table IV:- Characteristics of closely associated sequences in NCBI to sequences obtained from under-fives in UBTH, Benin City and NAUTH, Nnewi.

Accession no of close relatives in NCBI	Location	Source	Sequence name	Collection date	Serotypes
MK995001.1	Ethiopia	Faeces	Hexon protein gene	2016	AdV-40
MF962525.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962521.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962520.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962511.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962509.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962499.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962498.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962494.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40
MF962492.1	Mexico	Waste water	Hexon protein gene	2016	AdV-40

NCBI = National Centre for Biotechnology Information, MK995001.1 (MK = Locus name, 995001.1 = accession number and version), AdV-40 = Adenovirus serotype 40

The hexon proteins as obtained from the children attending the NnamdiAzikiwe University Teaching Hospital,Nnewi and University of Benin Teaching Hospital, Benin City upon homology modeling showed that the secondary structural features of hexon protein from human adenovirus, HAdVs (Benin City) were made up of helix and random coil (Figure 1a and 1b), while HAdVs (Nnewi) consisted only of random coils (Figure 2a and 2b).

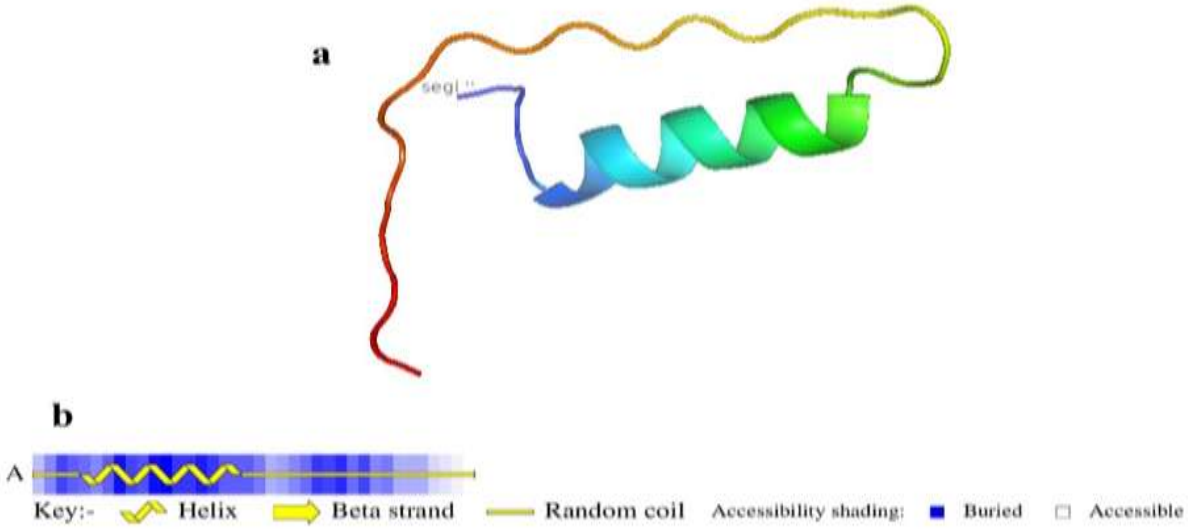

Figure 1:- (a) 3D structure of hexon protein of HAdVs (Benin City) (b) Secondary structural features of hexon protein of HAdVs (Benin City).

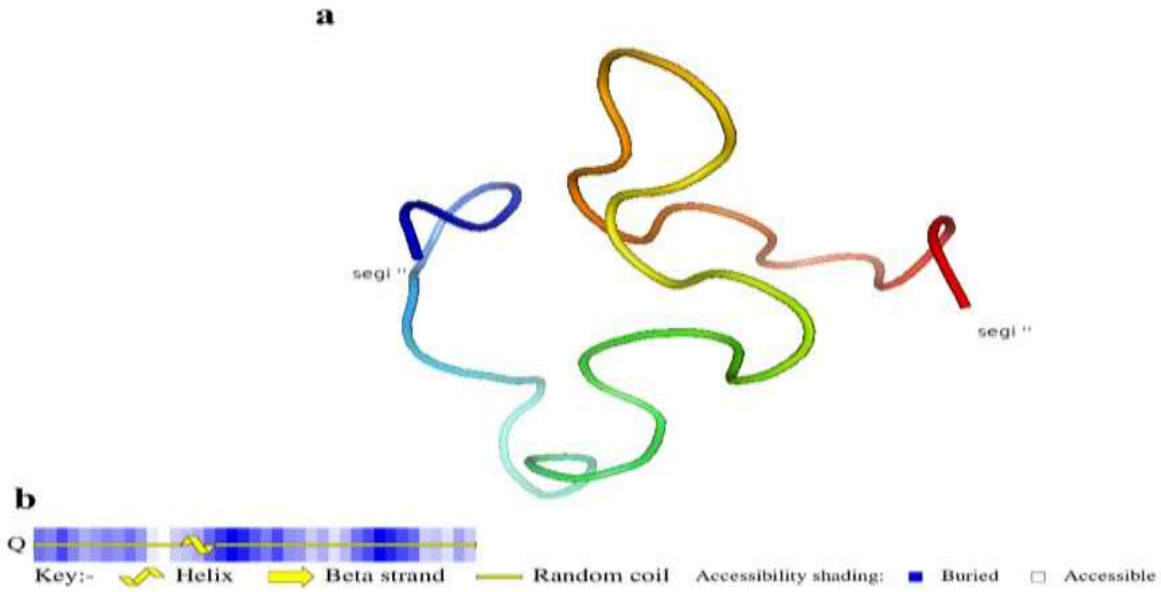

Figure 2:- (a) 3D structure of hexon protein of HAdVs (Nnewi) (b) Secondary structural features of hexon protein of HAdVs (Nnewi).

Results from using the Procheck server and Ramachandran plot to assess a protein's stereochemical quality(by examining the geometry of individual residues as well as the overall structural geometry),revealed that 92.6% and 75.9% of residues were in the most favourableareas of the hexon protein models of HAdVs (Benin City) and HAdVs (Nnewi), respectively, showing that they are dependable and high-quality models (Figure 3).

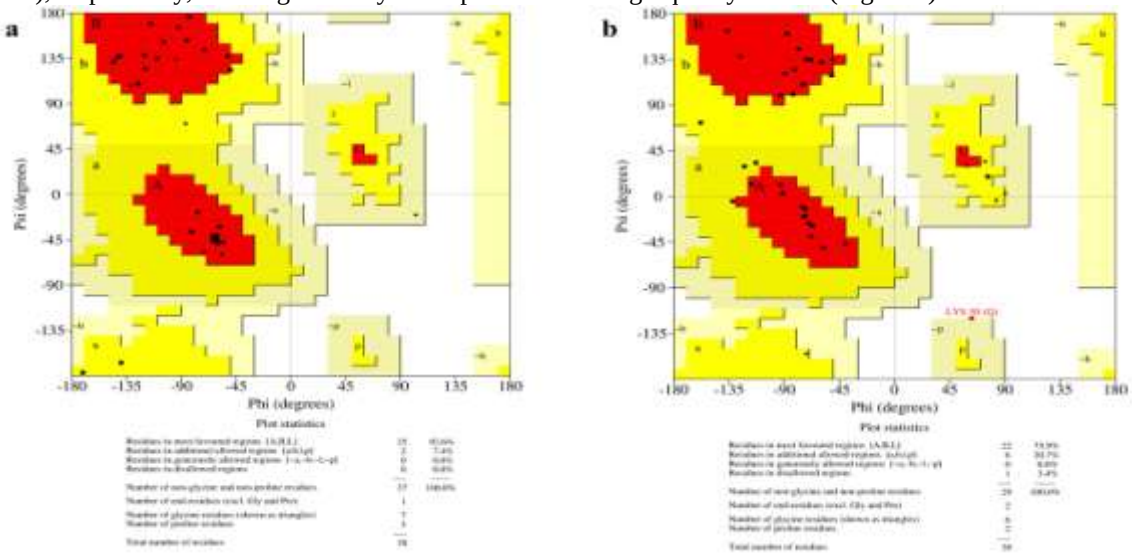

Figure 3:- 3D structural assessment based on Ramachandran plot (a) hexon protein HAdVs (Benin City) (b) hexon protein HAdVs (Nnewi).

Based on the hamming algorithm (an approximate distance in which a set of sequence differ), 4_Alfred-Adeno_E05_14 (Nnewi) and 10_Alfred-Adeno_C06_09 (Benin) were completely similar with hamming dissimilarity score of 0 and hamming similarity of 328, which is same as their individual sequence lengths (Tables 5 and 6). These two isolates appeared to be the most recent descent when compared to other isolates from both locations (Nnewi and Benin City) (Figure 4).

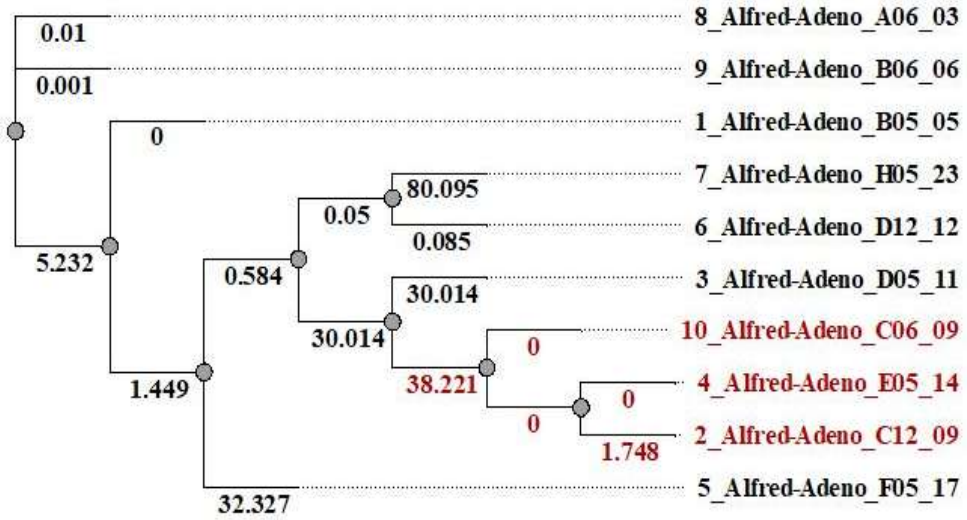

Figure 4:- Phylogram of the 16 sRNA showing the evolutionary relationship of the sequences obtained from isolatesof under-fives in NAUTH, Nnewi and UBTH, Benin City.

Table V:- Hamming dissimilarity index following multiple sequence alignment of the sequencesobtained from under-fives in Benin City and Nnewi.

	1_Alfred-Adeno_B05_05	2_Alfred-Adeno_C12_09	3_Alfred-Adeno_D05_11	4_Alfred-Adeno_E05_14	5_Alfred-Adeno_F05_17	6_Alfred-Adeno_D12_12	7_Alfred-Adeno_H05_23	8_Alfred-Adeno_A06_03	9_Alfred-Adeno_B06_06	10_Alfred-Adeno_C06_09
1_Alfred-Adeno_B05_05	0	227	232	239	232	188	223	224	225	239
2_Alfred-Adeno_C12_09	227	0	231	186	244	224	239	241	240	186
3_Alfred-Adeno_D05_11	232	231	0	221	282	230	241	265	261	221
4_Alfred-Adeno_E05_14	239	186	221	0	231	234	233	233	231	0
5_Alfred-Adeno_F05_17	232	244	282	231	0	242	248	249	248	231
6_Alfred-Adeno_D12_12	188	224	230	234	242	0	216	233	231	234
7_Alfred-Adeno_H05_23	223	239	241	233	248	216	0	260	260	233
8_Alfred-Adeno_A06_03	224	241	265	233	249	233	260	0	4	233
9_Alfred-Adeno_B06_06	225	240	261	231	248	231	260	4	0	231
10_Alfred-Adeno_C06_09	239	186	221	0	231	234	233	233	231	0

Table VI:- Hamming similarity index following multiple sequence alignment of the sequences obtained from under-fives in Benin City and Nnewi.

	1_Alfred-Adeno_B05_05	2_Alfred-Adeno_C12_09	3_Alfred-Adeno_D05_11	4_Alfred-Adeno_E05_14	5_Alfred-Adeno_F05_17	6_Alfred-Adeno_D12_12	7_Alfred-Adeno_H05_23	8_Alfred-Adeno_A06_03	9_Alfred-Adeno_B06_06	10_Alfred-Adeno_C06_09
1_Alfred-Adeno_B05_05	324	97	92	85	92	136	101	100	99	85
2_Alfred-Adeno_C12_09	97	332	101	142	88	108	93	91	92	142
3_Alfred-Adeno_D05_11	92	101	380	107	98	106	103	85	87	107
4_Alfred-Adeno_E05_14	85	142	107	328	97	94	95	95	97	328
5_Alfred-Adeno_F05_17	92	88	98	97	365	94	96	99	100	97
6_Alfred-Adeno_D12_12	136	108	106	94	94	336	120	103	105	94
7_Alfred-Adeno_H05_23	101	93	103	95	96	120	344	84	84	95
8_Alfred-Adeno_A06_03	100	91	85	95	99	103	84	348	344	95
9_Alfred-Adeno_B06_06	99	92	87	97	100	105	84	344	348	97
10_Alfred-Adeno_C06_09	85	142	107	328	97	94	95	95	97	328

Another set of closely related isolates were 8_Alfred-Adeno_A06_03 and 9_Alfred-Adeno_B06_06. These were both obtained from Nnewi and were found to be an out-group based on their phylogenetic assessments. Based on their evolutionary distance 9_Alfred-Adeno_B06_06 was observed to have evolved earlier than 8_Alfred-Adeno_A06_03 (Figure 4), with their respective hamming dissimilarity and similarity being 4 and 344 (Tables 5 and 6).

The adenoviruses, 4_Alfred-Adeno_E05_14 (Nnewi) and 10_Alfred-Adeno_C06_09 (Benin) were observed to be sharing a common ancestry and the same evolutionary distance (Figure 4).

Discussion:-

Adenoviruses are common viruses that can cause illness in humans. Most adenovirus infections cause mild respiratory illness, such as the common cold (Lynch and Kajon, 2016). Rotaviruses have been identified as predominantly the leading cause of diarrhoea in children who are under five years of age in our locality (Mathew et al., 2023). It may seem that the ease with which these children get infected with the rotavirus, as compared to other viruses, facilitates its pathogenesis and spread in that population.

In this study, the overall prevalence of adenovirus diarrhoea was 8.7%, with the diarrhoea being more prevalent in the females (55.2%) than the males (44.8%) studied from both locations, UBTH, Benin City and NAUTH, Nnewi. The higher prevalence of adenovirus diarrhoea in females than their male counterparts may be due partly to the anatomy of the female genital tract in which the urinary and anal openings have a close connection (Kumthip et al., 2019). On the other part, it is thought that the males have a 'perceived' stronger immunity than the females. Traditionally, in most parts of Africa including Nigeria, males are thought to be stronger because of their physique or body build up.

Earlier studies have documented varying reports presenting either sex as being more susceptible to adenovirus infection than the other (Mukhtar et al., 2015; Tagbo et al., 2014; Tagbo et al., 2019). However, while this controversy exists, it gives room for more studies to be done to establish whether the females are more prone to adenovirus infection than their male counterparts or vice-versa. The prevalence of adenovirus diarrhoea in this study is similar to an earlier report (Meryem et al., 2017), but lower than the 19.3% reported by (Imade and Eghafona, 2015). However, results from this study are higher than the report of a recent study done in Senegal which reported a prevalence of 3.1% among children who are under five years of age with diarrhea (Abdou et al., 2023), and that of (Tagbo et al., 2019), which reported a prevalence of 4.8% in some rural communities of Enugu State, Nigeria.

Transmission routes of adenoviruses include the faecal-oral route and inhalation of aerosols. Adenoviruses have been associated with outbreaks in different settings, including military camps (Du et al., 2021), hospitals (Kumthip et al., 2019), day care centers (Adeh et al., 2019) and schools (Biggs et al., 2018). Waterborne outbreaks due to adenoviruses have also involved those using swimming pools (Bonadonna et al., 2019).

A number of times, the infections caused by adenoviruses in children who are less than five years of age may be as a result of poor hygiene, sanitation, some form of carelessness by their mothers and feeding children with left-over foods which may have accumulated contaminants chiefly viruses for the period it was left unattended (Birhan et al., 2023). The implication of this is that such children end up becoming the reservoir for both viral and bacterial pathogens of diarrhoea, which if not checked could make them come down with severe diarrhea (Birhan et al., 2023).

None of the samples assayed from UBTH, Benin City and NAUTH, Nnewi using polymerase chain reaction tested positive for rotavirus diarrhoea, despite optimizing the PCR process and using well known primers (Meryem et al., 2017; Bonadonna et al., 2019). While earlier studies have shown that rotavirus is a leading cause of diarrhoea among children who are under five years of age in Nigeria (Ugboko et al., 2020, Crawford et al., 2017; Smith and Ajayi, 2017), the results of this study prove otherwise. A recent study done in Nigeria showed a pooled prevalence of 23% for rotavirus diarrhoea in children less than five years of age (Digwo et al., 2023). The result obtained from this study may have been because of the influence of rotavirus vaccine, Rotarix (GlaxoSmithKline Biologicals) which many of the parents had access to, even though it had not been introduced into the routine immunization schedule of the country as at the time of this study.

A recent study in Tanzania showed that although the Rotarix vaccine did not eliminate the rotavirus diarrhoea, it helped in reducing cases requiring hospitalization in that country (Hugho et al., 2023), agreeing with the results of this study. It has been reported by (Gbebangi-Manzemuet al., 2023)that rotavirus diarrhoea was only detected in 59 out of the 165 under-fives who had not been vaccinated in their recent study done in the Democratic Republic of Congo. This may have been the case with the current study as the results are consistent with theirs. In 2017, through the efforts of GlaxoSmithKline Biologicals and RotaTeq Merck, Rotarix and RotaTeq were introduced into the expanded programs on immunization (EPI) of African counties to provide protection against the harmful effects of rotavirus infection in children under five years of age (Smith and Ajayi, 2017).

In Nigeria, the Rotarix vaccine was officially introduced into the routine immunization programme in August 2022 with the aim of helping to gradually reduce rotavirus diarrhoea among under-fives in the country (Giwa, 2022). This study may perhaps be the first in southern Nigeria to report such a decline in the prevalence of rotavirus diarrhoea in children who are under five years of age.

Based on molecular detection, almost all the accessions (90%) obtained from children attending UBTH, Benin City and NAUTH, Nnewi matched largely with those obtained from waste water in Mexico. This may suggest that the transmission route for infection with human adenoviruses (HAdVs) in any of the study locations is through exposure

to dirty drinking water. Adenovirus infection may be caused by consumption of contaminated water or inhalation of aerosolized droplets during water recreation (Wang et al., 2021).

Following sequence annotation based on similarity with accessions in the Genbank, all the accessions obtained from children attending tertiary hospitals in Benin City and Nnewi were found to be hexon protein gene (Dirisu et al., 2023).The human adenovirus species with the lowest percentage similarity to its close relatives in Genbank was 98%, indicating related species. The species included in this investigation had pairwise nucleotide similarity values that fell within the range required for accurate species identificationand the sequence lengths were all annotated as hexon protein genes.The adenovirus (AdV) hexon constitutes the major virus capsid protein. The epitopes located on the hexon protein are targets of neutralizing antibodies in vivo, serve in the recognition by cytotoxic T cells, and provide the basis for the classification of adenoviruses into the 51 serotypes known to date (Lee et al., 2020).

In particular, the isolates obtained from children attending UBTH in Benin City and NAUTH, Nnewi matched largely with AdV-40 serotypes in the Genbank. Large studies using highly sensitive, type-specific molecular diagnostics have demonstrated a substantial and previously under-estimated burden of paediatricdiarrhoeal disease due to enteric infections with adenovirus types 40/41 (Sobolev et al., 2020).

The reliability and quality structure of the models were obtained using the Ramachandran plot, which is one of numerous evaluation techniques (Wlodawer, 2017). The Procheck server was used to assess a protein's stereochemical quality (Mohammadi-Kambs et al., 2017). Based on the Ramachandran plot, an excellent quality model is one that has more than 90% of its residues in the most favorable area (Wlodawer, 2017). Only hexon protein model from HAdVs (Benin City) had more than 90% of its residues in the best place, which showed that it is a very good model. Values of 95% or higher in Errat plot are typical for good high-resolution structures; therefore, the hexon protein model from HAdVs in Benin City is a good model.

Experimentally, an appropriate distance was determined, called minimum Hamming distance, in which the sequences of a set differ (Mohammadi-Kambs et al., 2017). Based on this algorithm, 4_Alfred-Adeno_E05_14 (from Nnewi) and 10_Alfred-Adeno_C06_09 (from Benin) were completely similar with hamming dissimilarity score of 0 and hamming similarity of 328, which is same as their individual sequence lengths. However, there seemed to be an isolate (2_Alfred-Adeno_C12_09) with an evolutionary distance of 1.748 which evolved from 4_Alfred-Adeno_E05_14 (from Nnewi), identified among children attending the tertiary health facility at Nnewi. This showed that there is a possible transmission of a newer strain of AdV-40 serotypes in Nnewi with a stronger infectivity than the previous. Evolution of viral pathogens may lead to altered virulence, enhanced transmission, altered tissue tropisms and striking new disease manifestations. As a result, understanding viral evolution is vital to predict and prevent future disease outbreaks. Adenoviruses, due to their broad tropism and tractability, offer a useful model for studying the molecular evolution of DNA viruses. Adenoviruses have played an invaluable role in the study of human biology; current paradigms for RNA splicing and viral oncogenesis are two such examples(Tessier et al., 2021). Human adenoviruses (HAdVs) are also significant agents of disease, ranging in severity from mild, self-limited infections of mucosal surfaces, to severe, life-threatening dissemination, particularly involving the respiratory tract (Adeh et al., 2019). A previous study suggested that outbreaks from evolving, novel HAdV types have been associated with fatal infections (Mathew et al., 2023).

Conclusion:-

This study concluded that the prevalence of adenovirus in the study locality is 8.7%, with more females (55.2%) infected than their male (44.8%) counterparts in children under five years of age. The study also showed that sex did not have any significant statistical effect on the spread of adenovirus infection in children less than five years of age ($p>0.05$; $\chi^2= 3.06$). There was no rotavirus genome targeted from among the samples used for this study by polymerase chain reaction.

Competing interests:
The authors declare no competing interests.

Funding:
The authors received no funding for this study.

Authors' contributions:
Dirisu JO, conceptualized the study and wrote the manuscript as part of his PhD thesis, Agbakoba NR supervised the entire research work, Okwelogu SI did the statistical analysis and proof reading, Eki-Udoko FE and Elo-Ilo JC diagnosed under-fives with diarrhoea in their paediatric clinics and helped with sample collection alongside Olukayode O.

Acknowledgements:-
The authors acknowledge the contribution of all the Medical Laboratory Scientists and Physicians in both study locations, as well as other staff who helped to make the study a success.

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